

after "IgG" insert --(Figure 6A) and controls (Figure 6C)--.

In the Claims

1. (Amended) A method for a [large-scale] production of a recombinant antigen-specific entire intact monoclonal antibody, said method comprising steps:

(a) selecting an antigen against which the specific antibody is to be produced and isolating, chemically synthesizing or amplifying with polymerase chain reaction (PCR) a cDNA, mRNA or genomic DNA [of genes for] encoding antibody light and heavy chains and assembling the antibody [genes] cDNA encoding said antibody light and heavy chains into two separate expression cassettes each cassette further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5' terminus and by the yeast transcription termination DNA sequence of the 3'-terminus [containing the cDNA];

(b) preparing a recombinant Pichia pastoris (P. pastoris) yeast expression vector pPICZ $\alpha$  by restriction digestion with EcoRI and BamHI;

(c) constructing a recombinant P. pastoris yeast expression plasmid containing the expression cassettes of step (a) [of cDNA of the light and heavy chain genes encoding the antibody];

(d) cloning the [antibody] expression cassettes of step (c) into the P. pastoris expression vector to generate recombinant plasmid pPICZ $\alpha$ LH;

(e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes of step (d) under the control of the AOX1 promoter fused to the DNA encoding the [a] *Saccharomyces cerevisiae*  $\alpha$ -mating factor signal [sequence];

(f) amplifying and isolating the recombinant plasmid;

(g) [preparing and] transforming *P. pastoris* spheroblasts with BglIII linearized, NotI linearized, SacI linearized, SalI linearized or StuI-linearized recombinant plasmid replacing the yeast chromosomal AOX1 DNA sequence with AOX1-antibody [gene] DNA sequence containing expression cassettes of the recombinant plasmid of step (d);

(h) selectively growing the recombinants;

(i) screening yeast transformation colonies for a recombinant antibody expression;

(j) analyzing putative positive yeast clones for chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;

(k) confirming the integrity of the DNA insert [or junction sequence];

(l) inducing the recombinant antibody expression;

(m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;

(n) detecting the presence of the recombinant antibody by Western blot; [and]

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ant  
(o) testing the recombinant antibody for specific antigen-antibody binding, and

(p) harvesting the antigen-specific antibody produced in steps (a) - (o).

2. (Amended) The method of claim 1 wherein the antibody [genes are] cDNA is assembled into the expression cassettes by subcloning the antibody light and heavy chain cDNA in tandem as EcoRI-BglIII/BsmBI fragments flanked by a DNA encoding the [a] *P. pastoris* signal sequence, preceded by a *P. pastoris* promoter at the 5'-terminus and by a P. pastoris yeast transcription termination DNA sequence at the 3'-terminus.

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3. (Amended) The method of claim 2 wherein the ~~signal~~ sequence is a yeast  $\alpha$ -factor and wherein the promoter is an alcohol oxidase AOX1-P.

4. (Amended) The method of claim 3 wherein the antigen is dioxin [yeast expression vector is pPICZ $\alpha$ ].

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5. (Amended) The method of claim 4 wherein the [yeast expression vector is prepared by restriction digestion with *EcoRI* and *BamHI*] antibody cDNA encoding the light and heavy chain is isolated from a hybridoma DB1 that recognizes dioxin.

6. (Amended) The method of claim 5 wherein the [recombinant plasmid is pPICZ $\alpha$ LH] light chain cDNA from the DD1 hybridoma comprises 666-bp and the heavy chain cDNA from the DD1 hybridoma comprises 1332-bp nucleotide sequence.

7. (Amended) The method of claim 6 wherein the recombinant [expression plasmid pPICZ $\alpha$ LH is constructed by cloning the antibody genes expression cassettes into the *P. pastoris* expression vector] anti-dioxin antibody is secreted into a supernatant.

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8. (Amended) The method of claim [7] 3 wherein the replacement of the yeast chromosomal AOX1 with AOX1-antibody [gene] cDNA containing cassettes is by homologous recombination replacement.

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9. (Amended) The method of claim 8 wherein the selective growth of the recombinants and elimination of non-recombinants is performed on a medium containing zeocin.

10. (Amended) The method of claim [9] 8 wherein the selective growth of the recombinants is performed on a medium containing g418, trimethoprin, or a compound that limits the growth of wild type *P. pastoris*.

Sub C3  
11. (Amended) The method of claim [10] 9 wherein the screening of transformed colonies for antibody expression is by colony-immunoblotting for the origin of the recombinant antibody.

12. (Amended) The method of claim 11 wherein the [screening] analysis of putative positive clones of step (j) is by a PCR or by a restriction analysis.

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13. (Amended) The method of claim 12 wherein the integrity of the cDNA inserts [or junction sequence] is confirmed by nucleotide sequence analysis.

Ant  
14. (Amended) A recombinant [Intact] antigen-specific monoclonal antibody [ies] produced by *Pichia pastoris* (*P. pastoris*) transformed with mouse, humanized mouse or human immunoglobulin DNA [genes], said antibody produced by the process comprising steps:

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(a) selecting an antigen against which the specific antibody is to be produced and isolating, chemically synthesizing or amplifying with polymerase chain reaction (PCR) a cDNA, mRNA or genomic DNA [of genes for] encoding antibody light and heavy chains and assembling the antibody [genes] cDNA encoding said antibody light and heavy chains into two separate expression cassettes, each cassette further comprising a flanking DNA signal sequence preceded by a yeast promoter at 5' terminus and by the yeast transcription termination DNA sequence of the 3'-terminus [containing the cDNA];

(b) [preparing] selecting a recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector pPICZ $\alpha$  by restriction digestion with EcoRI and BamHI;

(c) constructing a recombinant *P. pastoris* yeast expression plasmid containing the expression cassettes of step (a) [of cDNA of the light and heavy chain genes encoding the antibody];

(d) cloning the [antibody] expression cassettes of step (c) into the *P. pastoris* expression vector to generate recombinant plasmid pPICZ $\alpha$ LH;

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(e) transforming *Saccharomyces cerevisiae* with the recombinant plasmid by placing said expression cassettes of step (d) under the control of the AOX1 promoter fused to the DNA encoding the [a] *Saccharomyces cerevisiae*  $\alpha$ -mating factor signal [sequence];

(f) amplifying and isolating the recombinant plasmid;

(g) [preparing and] transforming *P. pastoris* spheroblasts with BglIII linearized, NotI linearized, SacI linearized, SalI linearized or StuI-linearized recombinant plasmid replacing the yeast chromosomal AOX1 DNA sequence with AOX1-antibody [gene] DNA containing expression cassettes of the recombinant plasmid of step (d);

(h) selectively growing the recombinants;

(i) screening yeast transformation colonies for a recombinant antibody expression;

(j) analyzing putative positive yeast clones for chromosomal integrates of the expression cassettes of heavy and light chain cDNAs;

(k) confirming the integrity of the DNA insert [or junction sequence];

(l) inducing the recombinant antibody expression;

(m) confirming the intactness of the expression cassettes inserts with PCR and Northern blot analysis;

(n) detecting the presence of the recombinant antibody by Western blot; [and]

(o) testing the recombinant antibody for specific antigen-antibody binding, and

(p) harvesting the antigen-specific antibody produced in steps (a) - (o).

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15. (Amended) The antibody of claim 14 wherein the antibody [genes are] cDNA is assembled into the expression cassettes by subcloning the antibody light and heavy chain cDNA in tandem as EcoRI-BglIII/BsmBI fragments flanked by a DNA encoding the P. pastoris signal sequence, preceded by a *P. pastoris* promoter at the 5'-terminus and by a *P. pastoris* yeast transcription termination DNA [sequence] at the 3'-terminus.

16. (Amended) The antibody of claim 15 produced by *P. pastoris* transformed with a human immunoglobulin cDNA [genes].

17. (Amended) The antibody of claim 15 produced by *P. pastoris* transformed with humanized mouse immunoglobulin cDNA [genes].

18. (Amended) The antibody of claim 15 produced by *P. pastoris* transformed with mammalian or mouse immunoglobulin cDNA [genes].

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19. (Amended) A recombinant *Pichia pastoris* (*P. pastoris*) yeast expression vector containing dual expression cassettes, each carrying an entire cDNA copy of immunoglobulin light and heavy chain DNA and further comprising a flanking signal DNA sequence preceded by a yeast promoter at 5'-terminus and by the yeast termination DNA sequence of the 3'-terminus.

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20. (Amended) An expression vector [system] comprising *Pichia pastoris* (*P. pastoris*) transformed with [antibody] human, mouse or humanized mouse immunoglobulin monoclonal cDNA [genes] for production of an entire recombinant antigen-specific intact antibody.

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21. (Amended) *Pichia pastoris* (*P. pastoris*) yeast transformed with expression cassettes carrying a cDNA [copy] of anti-dioxin immunoglobulin heavy and light chain [suitable for large-scale production of intact antibodies] isolated from DD1 hybridoma.